

1. Squash Multivirus resistance (MVR) Project

➤ Target:

GM Multivirus-resistance trait providing immunity to 4 major cucurbit virus groups of global economic importance in key squash producing regions.

➤ ssRNA Viruses:

- Cucumovirus (CMV)
- 3 Potyviruses (WMV2, ZYMV, PRSV)

➤ Project History:

- Initiated and led from Enkhuizen for ~10 years (vanGrinsven, deHaan, Gielen et al)
 - Early IP filings (virus-R & RNAi)
 - Group & collab's disbanded but some links remain
- Current project led from JH (IJE)
- Stage C - subject to intense review - decision on project continuation in Dec '03

FLXhif C

Squash MVR Approach & plans

MVR construct pZU684 ('4-in-1')



➤ Construct:

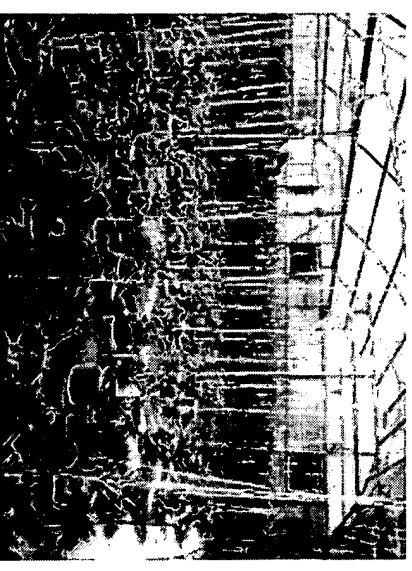
- Full QA + component report
- Unmodified virus sequences; ~600nt region of each virus partial ORFs
 - potyviruses = partial coat protein cistron + 3' non-translated region
 - CMV = 3' part of RNA2
- Cestrum promoter
- intron
- pmi selection (but inverted nos terms..)

➤ Current status:

83 putative transgenic lines in Naples, FL being testcrossed to generate seed for virus assays

➤ 2004 plan = take to proof of concept in squash:

- Recovery of testcross seed
 - Viral screening (x4) of testcross seed
 - Select events for progression (Stage C to D decision)
- Jan '04
- results at end April '04
- May '04



Squash MVR

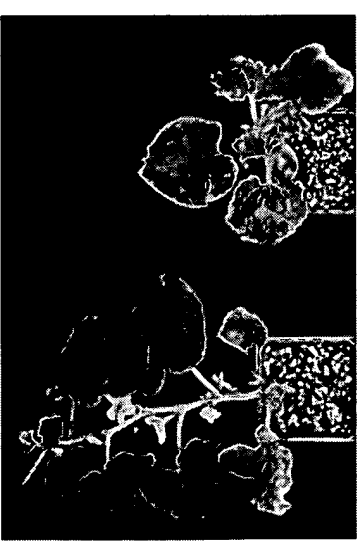
poc from elsewhere: Melon (Seeds)

> There is poc data for this construct in Melon (at seedling stage)

- Construct & Melon transgenics produced in Enkhuizen; Breeding & virus assays by Craig Sandlin, Seeds Gilroy, CA
- Line 684-42 shows good resistance to ZYMV, PRSV & CMV [WMV screen underway]
 - good segregation & correlation of phenotype with presence of transgene
- Line 684-12 also shows some resistance

Table 1. Summary of the ZYMV inoculation results. Blue shading indicates lines that appear to be resistant.

LINE	TOTAL TESTED	# RESISTANT	# SUSCEPTIBLE	EXPECTED RATIO
M024 - Inoculated	54	0	54	0:1
M024 - Noninoculated	18	18	0	1:0
684-2B Selfed	12	0	12	3:1
684-6A Selfed	12	0	12	3:1
684-12A Selfed	11	8	3	3:1
684-13C Selfed	15	1	14	3:1
684-22A Selfed	15	2	13	3:1
684-39A Selfed	7	0	7	3:1
684-39B Selfed	6	0	6	3:1
M024 X 684-39A	14	0	14	1:1
684-40C Selfed	15	0	15	3:1
684-42B Selfed	15	11	4	3:1
M024 X 684-42A	15	8	7	1:1
684-45A Selfed	15	0	15	3:1



Melon transgenic line pZU684-42B
PRSV inoculation

Line 684-12 shows some resistance

Line 684-42 shows good resistance to ZYMV, PRSV & CMV
– good correlation of phenotype & presence of transgene

Data is forwarded to IP (Mary K) upon receipt

syngenta